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Sakamoto et al.

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(54) **URINE SAMPLE ANALYZING METHOD AND SAMPLE ANALYZER INCLUDING CLASSIFYING EPITHELIAL CELLS INTO AT LEAST TWO TYPES BASED ON THE CHANGE OF POLARIZATION CONDITION**

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G01J 1/04	(2006.01)
G01J 4/00	(2006.01)
G01N 21/53	(2006.01)
G01N 21/64	(2006.01)
G01N 15/14	(2006.01)

(52) **U.S. Cl.**

CPC ***G01N 33/493*** (2013.01); ***G01J 1/0429*** (2013.01); ***G01J 4/00*** (2013.01); ***G01N 15/1434*** (2013.01); ***G01N 21/21*** (2013.01); ***G01N 21/53*** (2013.01); ***G01N 21/6486*** (2013.01); ***G01N 33/5005*** (2013.01)

(58) **Field of Classification Search**

CPC G01N 21/21; G01N 2021/0346; G01N 21/6486; G01N 33/493; G01N 30/74

USPC 250/225, 222.2, 573, 576; 435/34, 435/288.7, 31; 356/246, 73, 317, 327, 322

See application file for complete search history.

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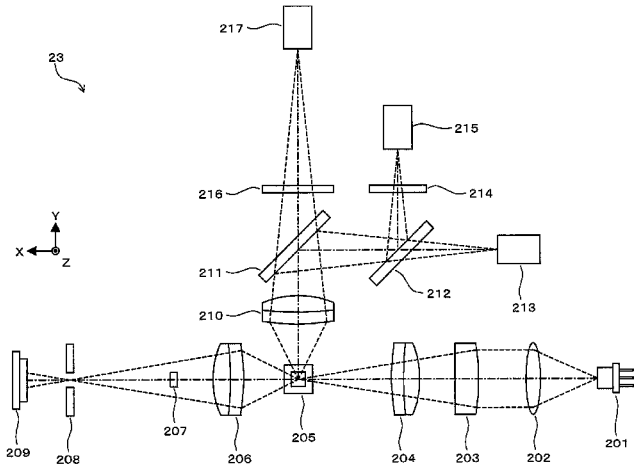
(57) **ABSTRACT**

Disclosed is a urine sample analyzing method comprising: flowing a measurement specimen prepared by mixing a urine sample and reagent through a flow cell; irradiating epithelial cells in the measurement specimen flowing through the flow cell with linearly polarized light and thereby producing scattered light; detecting a change of polarization condition of the scattered light produced by each of the epithelial cells; and classifying the epithelial cells into at least two types based on the change of polarization condition.

20 Claims, 9 Drawing Sheets



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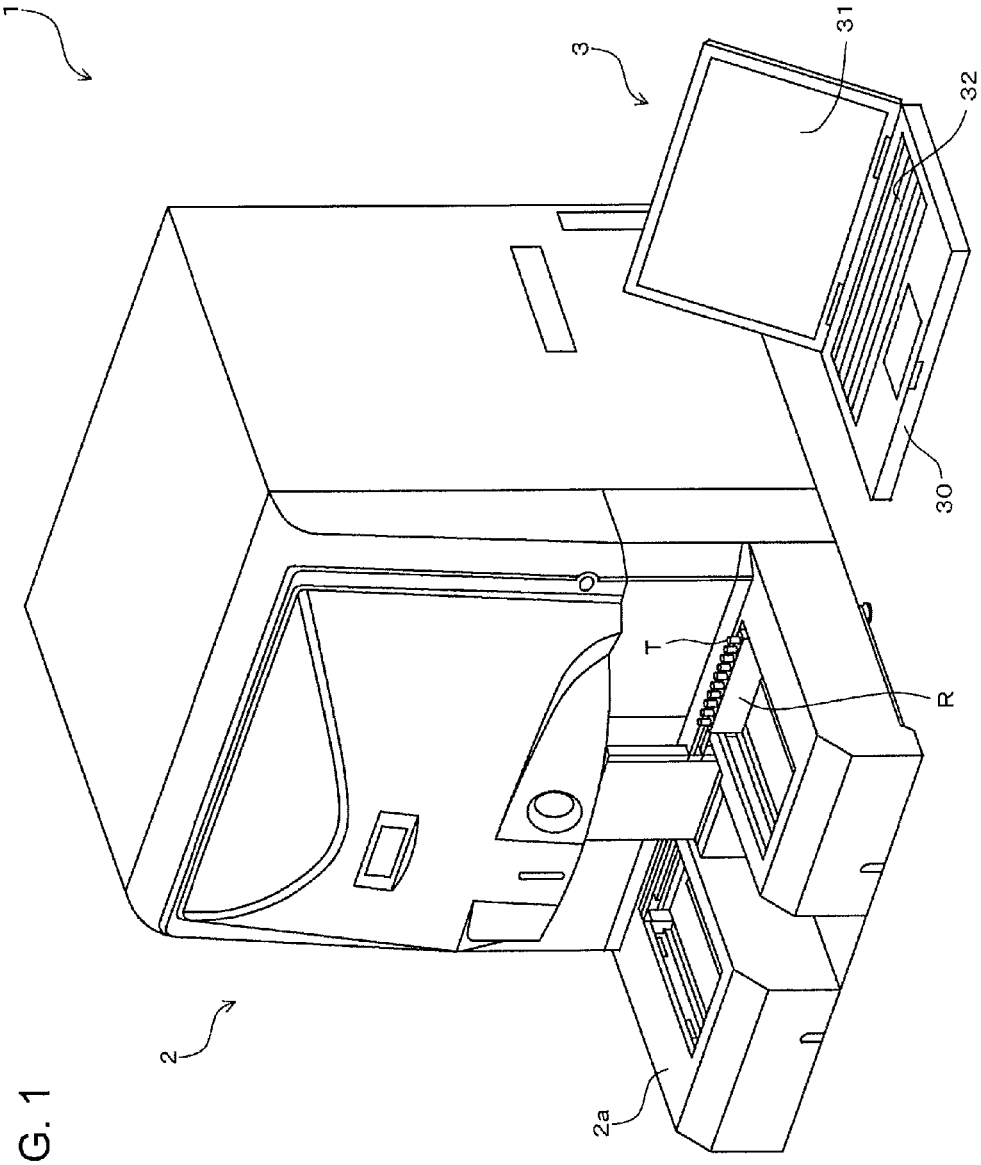
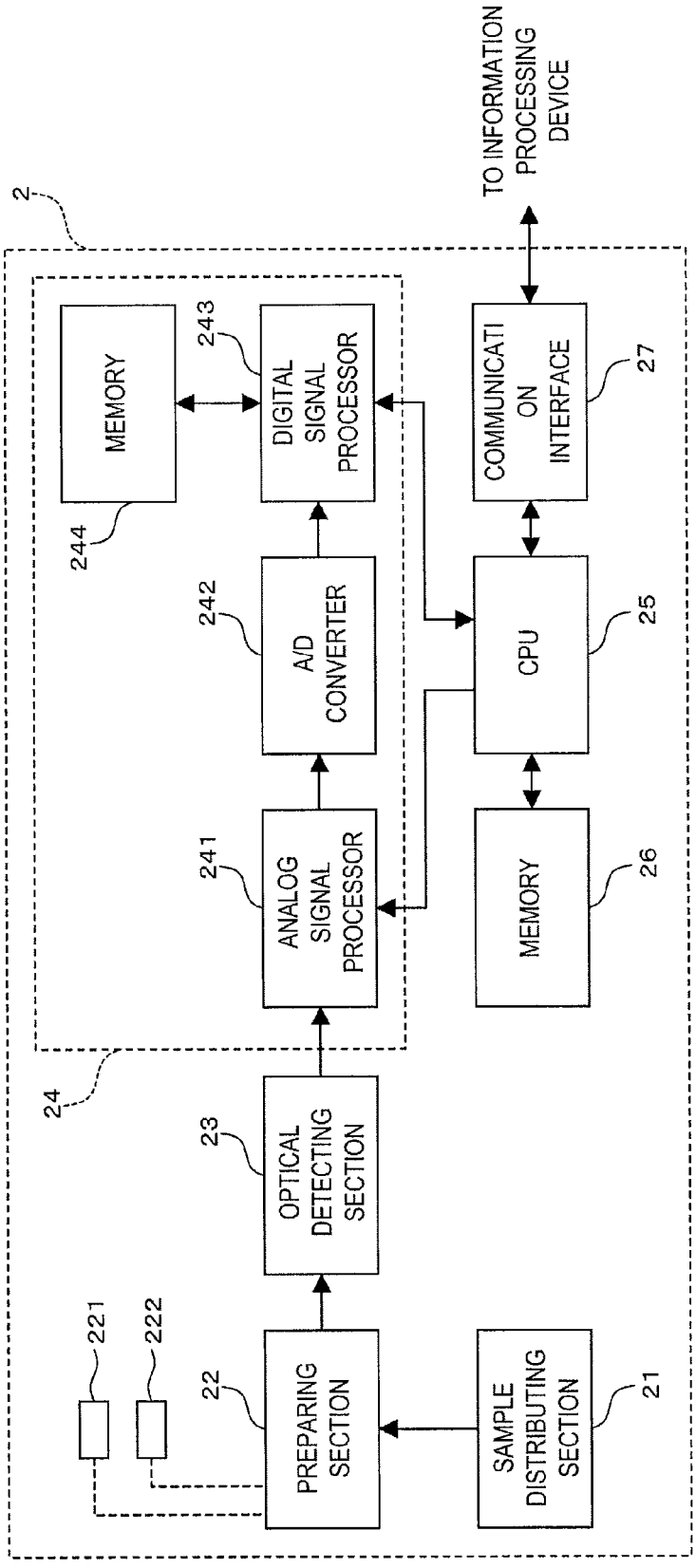


FIG. 1

FIG. 2


TO INFORMATION PROCESSING DEVICE
2
MEMORY
243
DIGITAL SIGNAL PROCESSOR
COMMUNICATION INTERFACE
27
244
242
A/D CONVERTER
CPU
25
241
ANALOG SIGNAL PROCESSOR
MEMORY
26
24
23
OPTICAL DETECTING SECTION
221
222
22
PREPARING SECTION
SAMPLE DISTRIBUTING SECTION
21

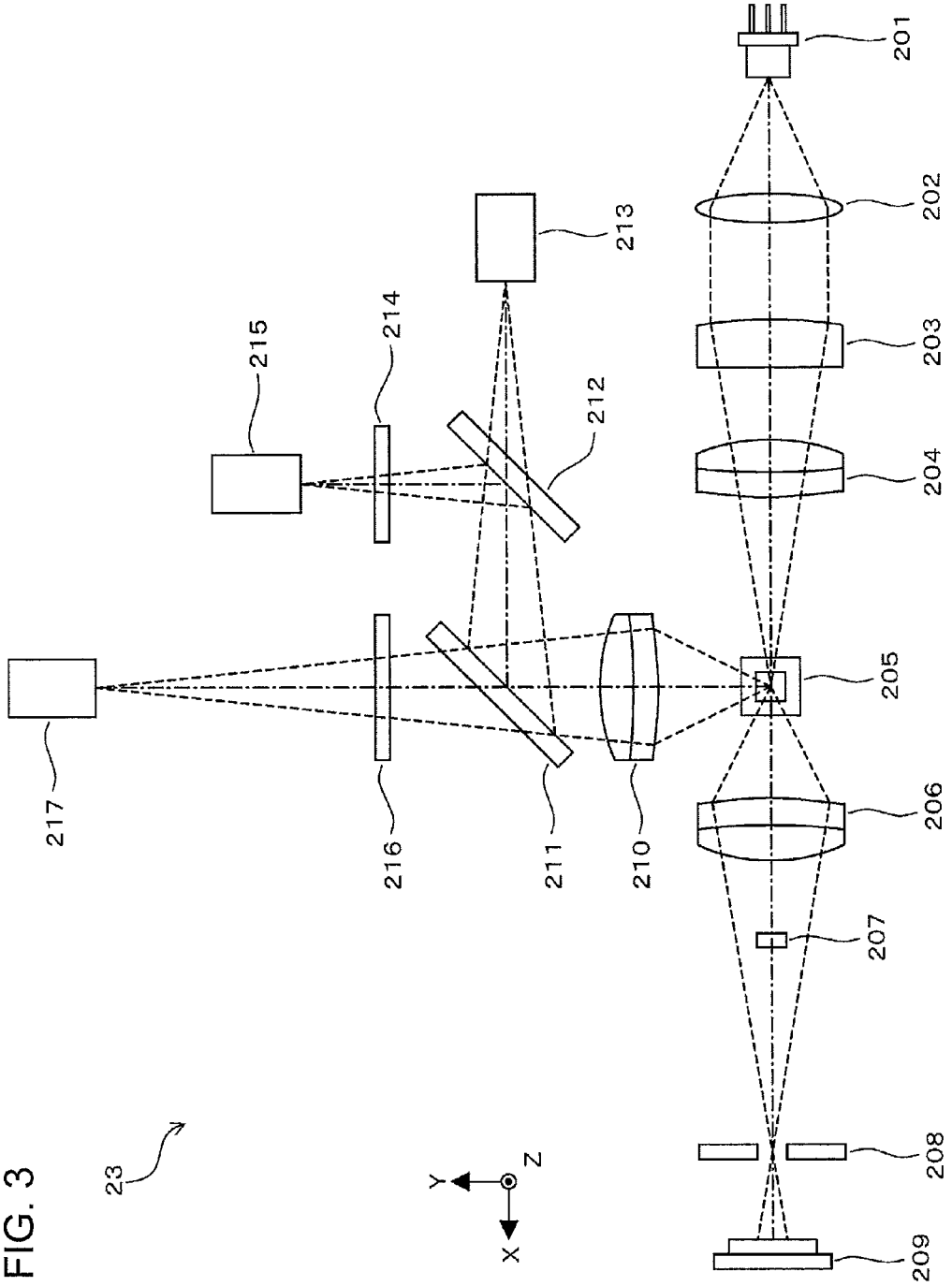

201
202
213
214
215
203
212
204
205
217
216
211
210
206
207
208
209
23
Y
Z
X

FIG. 3

FIG. 4A

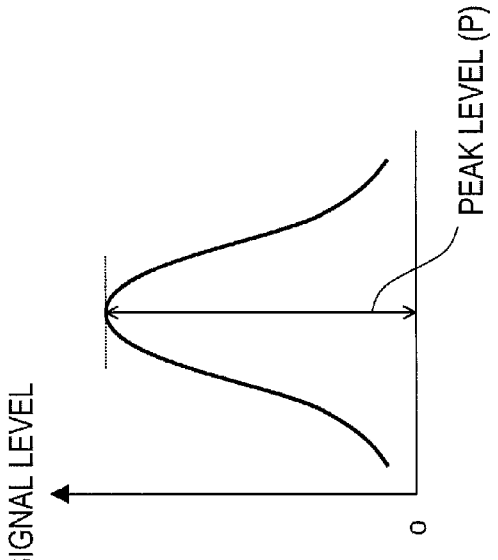


FIG. 4B

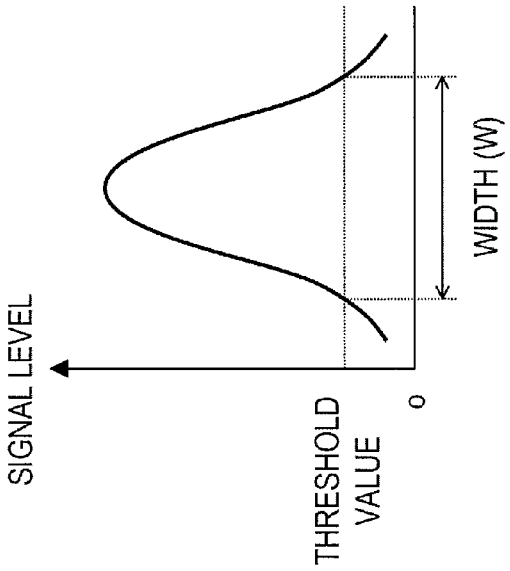
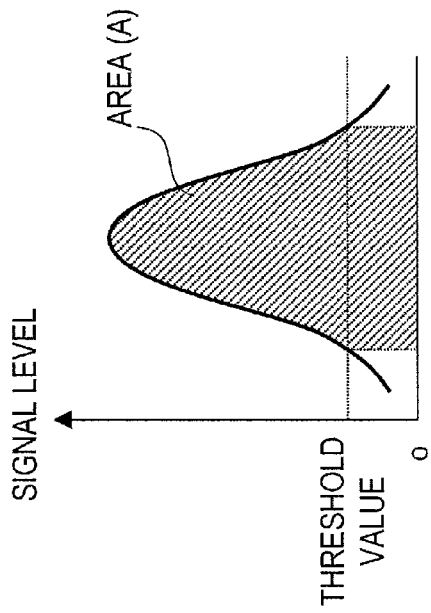


FIG. 4C



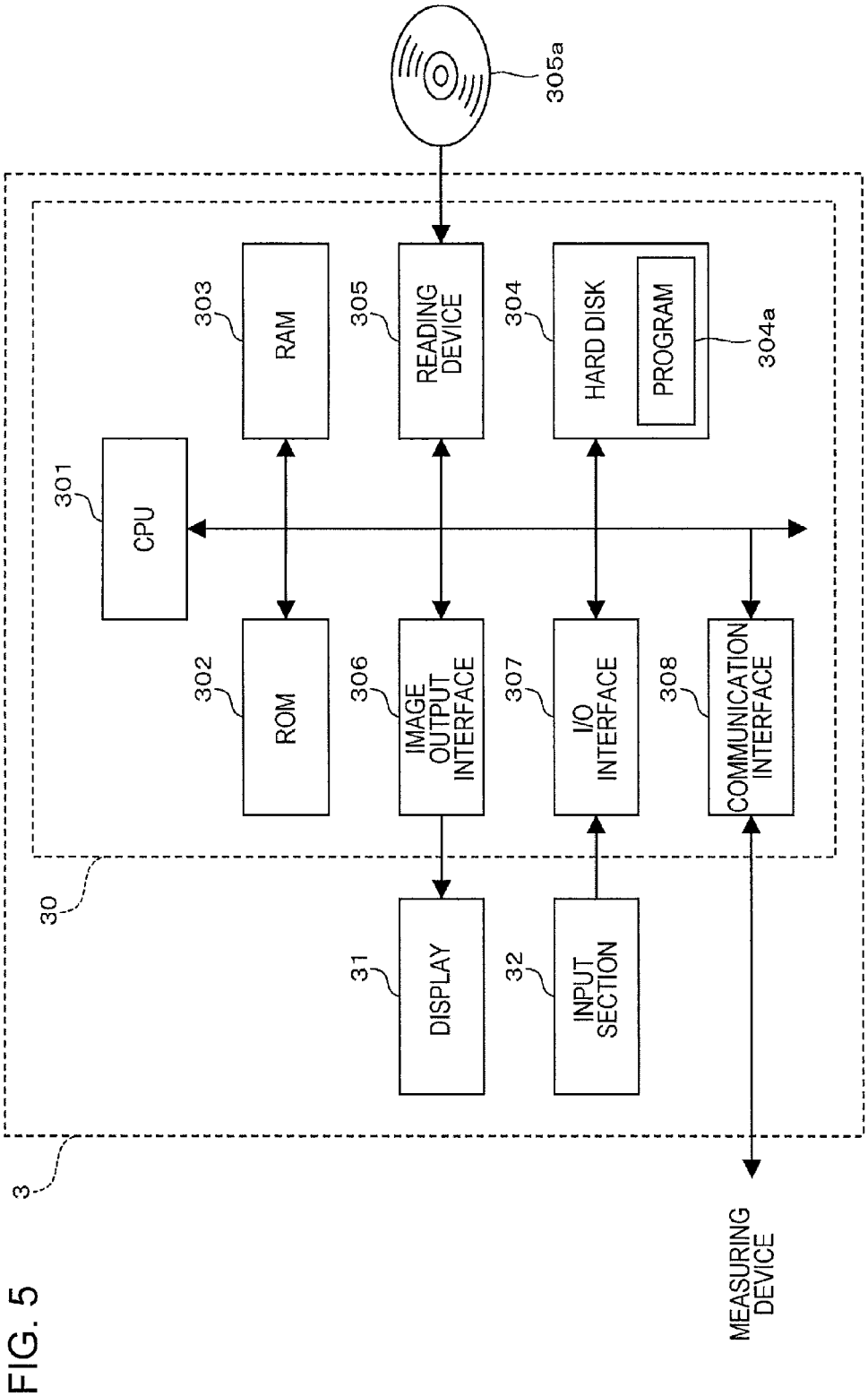

305a
303
RAM
305
READING DEVICE
304
HARD DISK
PROGRAM
304a
301
CPU
302
ROM
306
IMAGE OUTPUT INTERFACE
307
I/O INTERFACE
308
COMMUNICATION INTERFACE
30
31
DISPLAY
32
INPUT SECTION
3
MEASURING DEVICE

FIG. 5

FIG. 6

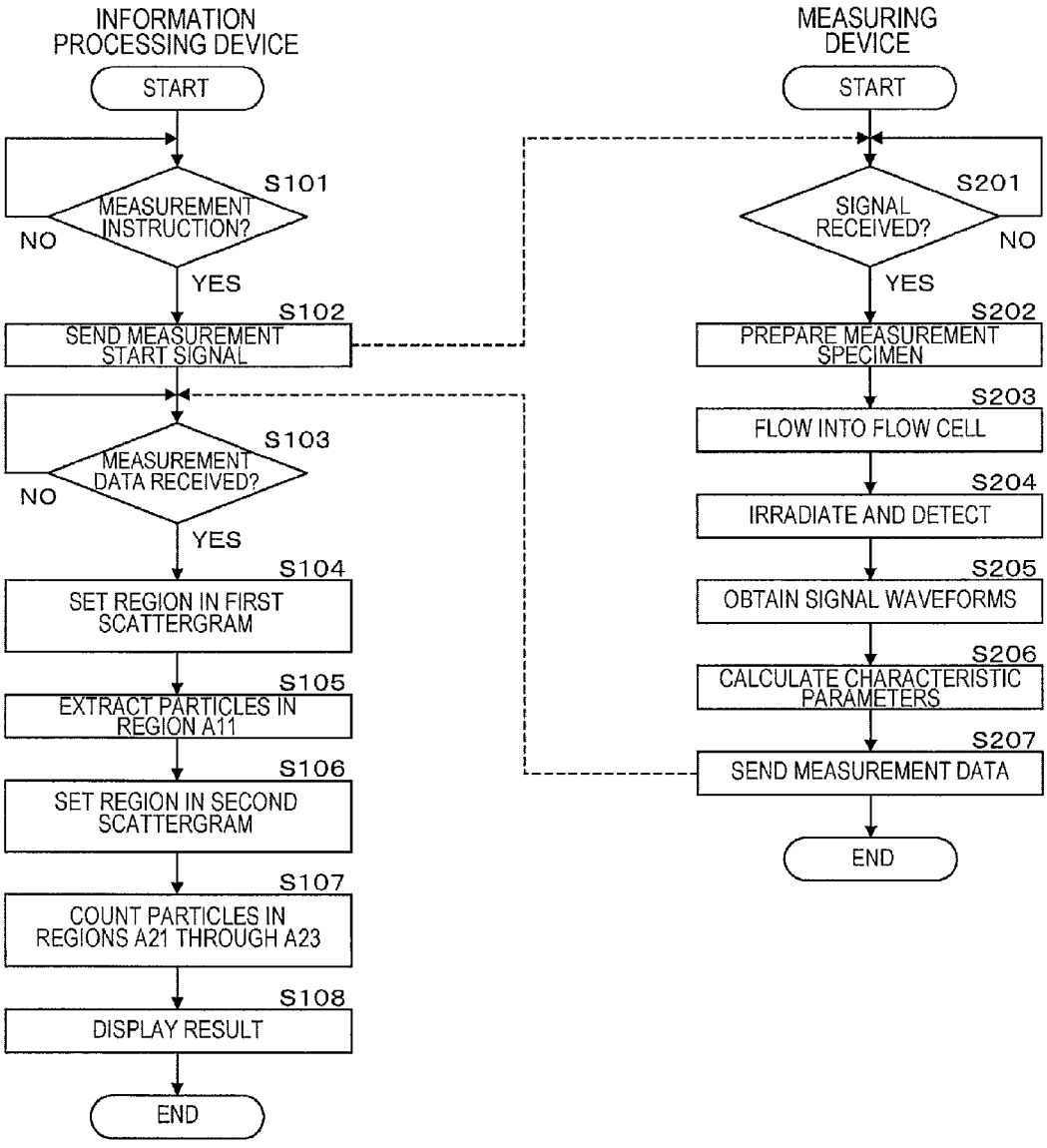

INFORMATION PROCESSING DEVICE
START
S101
MEASUREMENT INSTRUCTION?
NO
YES
S102
SEND MEASUREMENT START SIGNAL
S103
MEASUREMENT DATA RECEIVED?
NO
YES
S104
SET REGION IN FIRST SCATTERGRAM
S105
EXTRACT PARTICLES IN REGION A11
S106
SET REGION IN SECOND SCATTERGRAM
S107
COUNT PARTICLES IN REGIONS A21 THROUGH A23
S108
DISPLAY RESULT
END
MEASURING DEVICE
START
S201
SIGNAL RECEIVED?
NO
YES
S202
PREPARE MEASUREMENT SPECIMEN
S203
FLOW INTO FLOW CELL
S204
IRRADIATE AND DETECT
S205
OBTAIN SIGNAL WAVEFORMS
S206
CALCULATE CHARACTERISTIC PARAMETERS
S207
SEND MEASUREMENT DATA
END

FIG. 7B

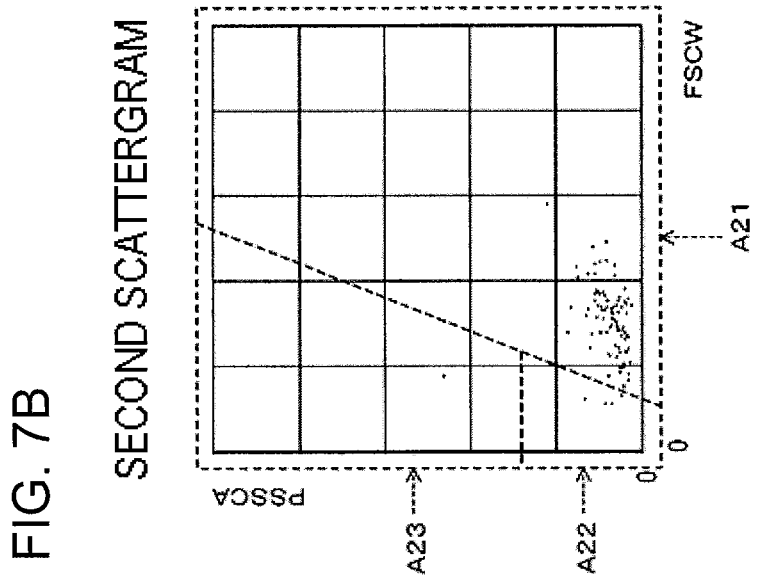

SECOND SCATTERGRAM
FSCW
A21
PSSCA
A23
A22
0
0

FIG. 7A

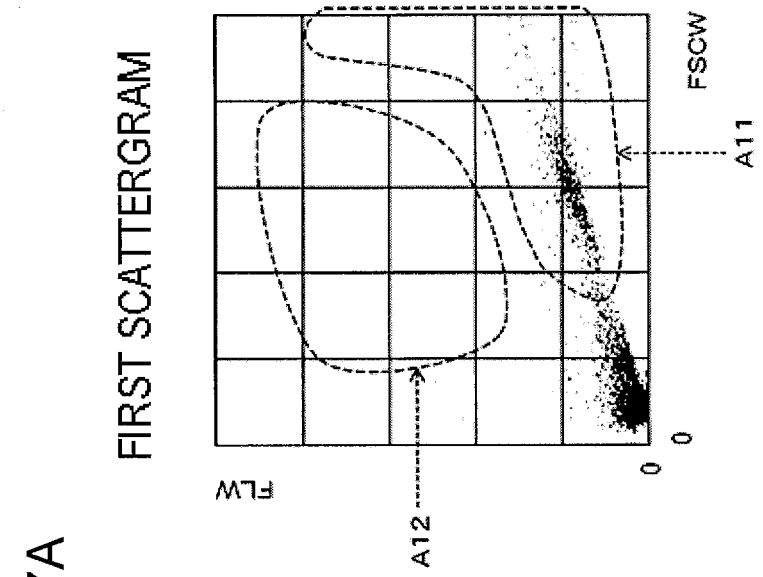

FIRST SCATTERGRAM
FSCW
A11
FLW
A12
0
0

FIG. 8A

	Visual	Embodiment
Squamous epithelial cells	14.0 / μL	11.9 / μL
Renal tubular epithelial cells	0.0 / μL	0.6 / μL
Ovoid fat bodies	0.0 / μL	0.0 / μL

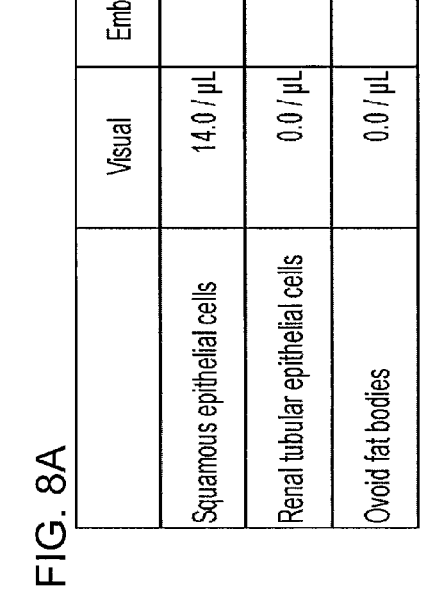


FIG. 8B

FIG. 8C

	Visual	Embodiment
Squamous epithelial cells	26.0 / μL	12.3 / μL
Renal tubular epithelial cells	42.0 / μL	35.0 / μL
Ovoid fat bodies	0.0 / μL	0.0 / μL

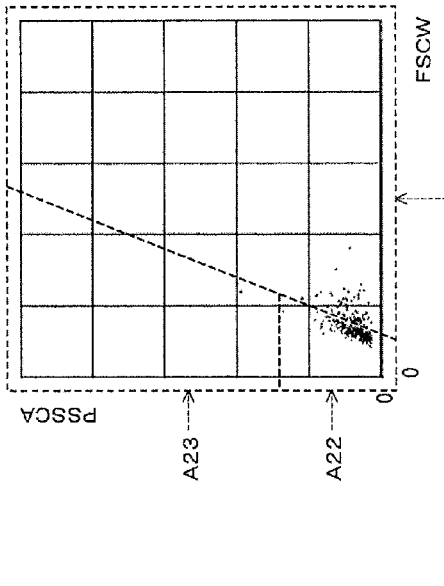


FIG. 8D

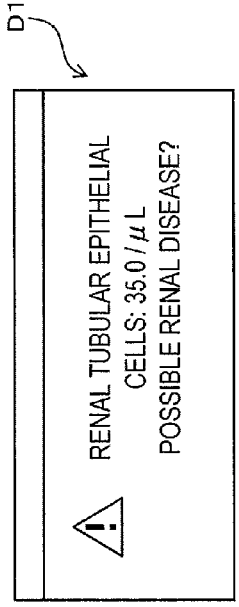


FIG. 9A

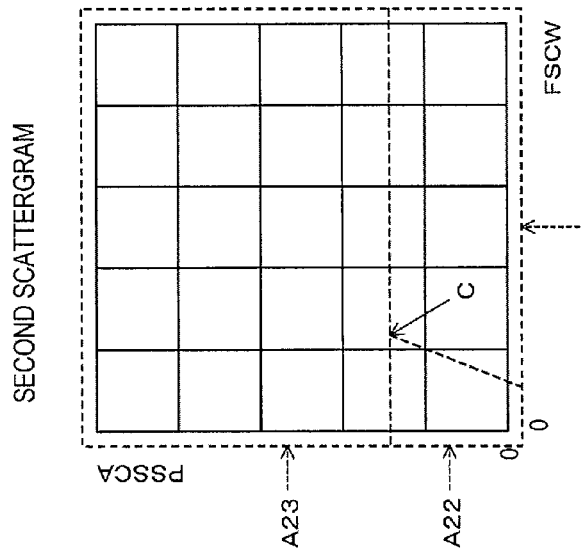


FIG. 9B

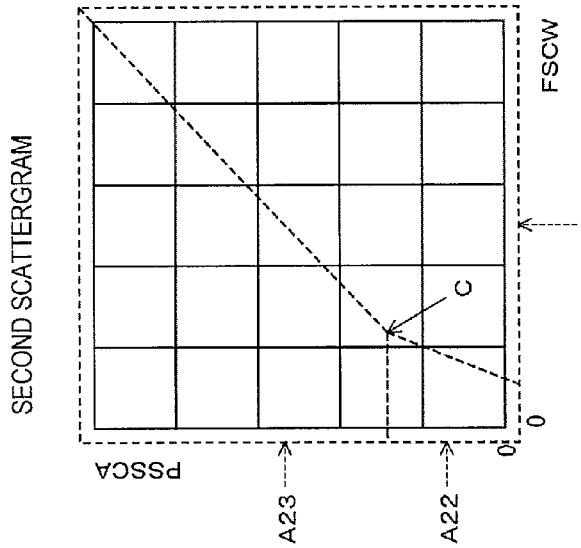


FIG. 9C

URINE SAMPLE ANALYZING METHOD AND SAMPLE ANALYZER INCLUDING CLASSIFYING EPITHELIAL CELLS INTO AT LEAST TWO TYPES BASED ON THE CHANGE OF POLARIZATION CONDITION

RELATED APPLICATIONS

This application claims priority under 35 U.S.C. §119 to Japanese Patent Application No. 2013-178938 filed on Aug. 30, 2013, the entire content of which is hereby incorporated by reference.

FIELD OF THE INVENTION

The present invention relates to a urine sample analyzing method and sample analyzer for analyzing samples.

BACKGROUND OF THE INVENTION

There is known conventional art for detecting particles in urine samples using a flow cytometer. For example, Japanese Laid-open Patent No. 2006-17555 discloses art for classifying the two groups of surface layer squamous type epithelial cells and epithelial cells other than the surface layer squamous type in urine by combining several parameters such as forward scattered light and fluorescent light from particles in a sample using a flow cytometer.

According to that, epithelial cells other than surface layer squamous type epithelial cells, may appear in cases of inflammation and disease, and those are seldom observed in urine samples of healthy individuals. On the other hand, surface layer squamous type epithelial cells are plentiful in urine samples of healthy persons.

Also according to the above document, parameters can be established for estimating the presence/absence of inflammation and disease in a patient who provided a urine sample by calculating the percentage of epithelial cells other than surface layer squamous type epithelial cells relative to the total number of epithelial cells.

Urine samples in cases of suspected disease or inflammation may require detailed information extraction to diagnose the suspected disease or abnormality of the urinary organs. There are several types of epithelial cells of different origin, including squamous epithelial cells, renal tubular epithelial cells, ovoid fat body and the like, which are useful in identifying the origin site of disease and inflammation if the type of epithelial cell contained in the urine sample can be identified.

SUMMARY OF THE INVENTION

The scope of the present invention is defined solely by the appended claims, and is not affected to any degree by the statements within this summary.

A first aspect of the present invention is a urine sample analyzing method comprising: flowing a measurement specimen prepared by mixing a urine sample and reagent through a flow cell; irradiating epithelial cells in the measurement specimen flowing through the flow cell with linearly polarized light and thereby producing scattered light; detecting a change of polarization condition of the scattered light produced by each of the epithelial cells; and classifying the epithelial cells into at least two types based on the change of polarization condition.

A second aspect of the present invention is a urine sample analyzer comprising a preparing section that prepares a measurement specimen by mixing a urine sample and reagent; a flow cell through that flows the measurement specimen prepared by the preparing section; an optical detecting section that irradiates linearly polarized light on particles in the measurement specimen flowing through the flow cell to produce scattered light, and detect a change of polarization condition of the scattered light produced by each of the epithelial cells; and a computer programmed to classify epithelial cells contained in the measurement specimen into at least two types based on the change of the polarization condition

A sample analyzing method comprising forming a sample flow of a measurement specimen containing epithelial cells; irradiating the sample flow with a linearly polarized light to trigger a polarization scrambling; and classifying the epithelial cells into at least squamous epithelial cells and other type of epithelial cells based on a degree of the polarization scrambling.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. **1** shows an external view of an embodiment of the urine sample analyzer;

FIG. **2** shows the structure of the measuring device of the embodiment;

FIG. **3** shows the structure of the optical detecting device of the embodiment;

FIGS. **4**A through **4**C illustrate the characteristic parameters of the embodiment. Specifically, FIG. **4**A illustrates a peak level; FIG. **4**B illustrates a width; and FIG. **4**C illustrates an area;

FIG. **5** shows the structure of the information processing device of the embodiment;

FIG. **6** is a flow chart showing the processes of the measuring device and information processing device of the embodiment;

FIG. **7**A shows a first scattergram and a region set in the first scattergram;

FIG. **7**B shows a second scattergram and a region set in the second scattergram of the embodiment;

FIG. **8**A shows comparative results of the visual count results obtained by microscope and results obtained by the embodiment;

FIG. **8**B shows the second scattergram obtained by the embodiment;

FIG. **8**C shows comparative results of the visual count results obtained by microscope and results obtained by the embodiment;

FIG. **8**D shows the second scattergram obtained by the embodiment;

FIG. **9**A shows a screen shown on the display;

FIG. **9**B shows the region set in the second scattergram in a modification; and

FIG. **9**C shows the region set in the second scattergram in a modification.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

The present embodiment is a urine sample analyzer for analyzing urine samples which contain particles such as blood cells, bacteria, casts, and epithelial cells. The urine samples to be measured include eliminated urine from a living body, secreted urine, primitive urine, urine of the urinary tract, urine in the bladder, and urine in the urethra.

The embodiment is described below with reference to the drawings.

FIG. **1** shows the exterior structure of a urine sample analyzer **1**.

The urine sample analyzer **1** has a measuring device **2** for optically measuring particles contained in the urine sample via flow cytometer, and an information processing device **3** for processing the measurement data output from the measuring device **2**. A transporting unit **2***a* is provided in front of the measuring device **2**, and the rack R holding a plurality of containers T containing urine samples is moved by the transporting unit **2***a*. The information processing device **3** is provided with a main body **30**, display **31** for displaying analysis results and the like, and an input unit **32** for receiving instructions from the operator.

FIG. **2** shows the structure of the measuring device **2**.

The measuring device **2** includes a sample distributing section **21**, a preparing section **22**, optical detecting section **23**, signal processing section **24**, CPU **25**, memory **26**, and communication interface **27**. The signal processing section **24** has an analog signal processor **241**, A/D converter **242**, digital signal processor **243**, and memory **244**.

The sample distributing section **21** aspirates a predetermined amount of urine sample from the container T transported by the transporting unit **2***a*, and supplies the aspirated sample to the preparing section **22**. The preparing section **22** has a mixing chamber and a pump (not shown in the drawings). Containers **221** and **222** are connected through a tube to the preparing section **22**. The container **221** contains reagent that includes intercalator for specifically staining nucleic acid, and container **222** contains diluting liquid. The sample supplied from the sample distributing section **21** into the mixing chamber is mixed with diluting liquid and reagent from the containers **221** and **222** to prepare the measurement specimen. The measurement specimen prepared in the mixing chamber is supplied together with sheath fluid to a flow cell **205** (refer to FIG. **3**) of the optical detecting section **23**.

FIG. **3** is a schematic view showing the structure of the optical detecting section **23**.

The optical detection section **23** includes a light source **201**, collimator lens **202**, cylindrical lens **203**, condenser lens **204**, flow cell **205**, collecting lens **206**, beam stopper **207**, pinhole **208**, FSC detector **209**, collecting lens **210**, dichroic mirror **211**, half mirror **212**, SSC detector **213**, polarization filter **214**, PSSC detector **215**, spectral filter **216**, and SFL detector **217**.

The light source **201** emits laser light having an approximate wavelength of 488 nm in the X-axis positive direction. The laser light emitted from the light source **201** is linearly polarized light. The light source **201** is arranged within the measuring device **2** so that the polarization direction of the linearly polarized light is parallel to the direct (Z-axis direction) of the flow of the measurement specimen running in the flow cell **205**. That is, the polarization direction of the light emitted from the light source **201** is perpendicular to the incidence surface when the incidence surface is perpendicular to the Z-axis direction.

The laser light from the light source **201** is converted to parallel rays by the collimator lens **202**. The laser light that passes through the collimator lens **202** is converged only in the Y-axis direction by the cylindrical lens **203**. The laser light that passes through the cylindrical lens **203** is collected in the Y-axis direction and Z-axis direction by the condenser lens **204**. Hence, the laser light emitted from the light source **201** irradiates a beam narrow along the Y-axis direction on the measurement specimen flowing in the Z-axis direction within the flow cell **205**. When the laser light irradiates particles in the measurement specimen, forward scattered light is produced in the forward direction (X-axis positive direction) of the flow cell **205**, and side scattered light and side fluorescent light are produced in a lateral direction (Y-axis positive direction) of the flow cell **205**.

The forward scattered light is collected at the position of the pinhole **208** by the collecting lens **206** arranged on the X-axis positive direction side of the flow cell **205**. Among the light emitted from the light source **201**, the laser light that passes through the flow cell **205** without illuminating particles in the measurement specimen is collected by the collecting lens **206**, and then is blocked by the beam stopper **207** so as to not impinge the detector **209**. The forward scattered light that passes through the pinhole **208** is detected by the FSC detector **209**. The detector **209** outputs a forward scattered light signal (FSC) based on the detected forward scattered light.

The side scattered light is converged by the collecting lens **210** arranged on the Y-axis positive direction side of the flow cell **205**. The side scattered light that passes through the collecting lens **210** is reflected by the dichroic mirror **211**. A part of the side scattered light reflected by the dichroic mirror **211** is split by the non-polarizing type half mirror **212**. The side scattered light that passes through the half mirror **212** is detected by the SSC detector **213**. The SSC detector **213** outputs a side scattered light signal (SSC) based on the detected side scattered light. Another part of the side scattered light reflected by the half mirror **212** impinges the polarization filter **214**.

When polarized laser light is irradiated on particles in the measurement specimen, the polarization direction of the side scattered light changes according to the optical rotating power of the component contained in the particle. In the present embodiment, the polarization direction of the laser light irradiating the particles in the measurement specimen is parallel to the flow direction (Z-axis direction) of the measurement specimen flowing through the flow cell **205** (hereinafter, this polarization condition is referred to as the "initial polarization condition"). When the laser light is irradiated to the measurement specimen, the polarization direction of the laser light rotates to a polarization direction that differs from the initial polarization condition. As the polarization of the laser light is partially scrambled by irradiation on particles, the side scattered light produced in the Y-axis positive direction includes rays of various polarization conditions.

Among the rays of the side scattered light produced from the particles, the percentage of rays polarized perpendicular to the initial polarization direction, that is, degree of polarization scrambling, is determined according to the components contained in the particles. As previously described, in the present embodiment several types of epithelial cells are observed contained in the solid components with different inherent polarization characteristics according to type, and the several types of epithelial cells can be classified based on the polarization condition of the side scattered light.

The polarization filter **214** is configured to block the polarized light parallel to the Z-axis direction, and transmit the polarized light parallel to the X-axis direction. The side scattered light that has passed through the polarization filter **214** is referred to as "polarization scrambled side scattered light" or "PSSC light" hereinafter. The polarization scrambled side scattered light is detected by the PSSC detector **215**. The PSSC detector **215** outputs a polarization scrambled side scattered light signal (PSSC) based on the detected polarization scrambled side scattered light.

As previously mentioned, the polarization direction of the side scattered light changes from the initial polarization condition according to the optical rotating power possessed by the particle in the measurement specimen. Therefore, the

amount of PSSC light that reaches the PSSC detector **215** also differs for each type of particle irradiated by the laser light, and the magnitude of the PSSC signal also differs for each type of particle irradiated by the laser light.

The forward scattered light emitted from the flow cell **205** and the side scattered light passed through the half mirror **212** are directly received by the FSC detector **209** and SSC detector **213**, respectively, and do not pass through a polarization filter. Therefore, the FSC detector **209** detects the forward scattered light including rays of nonuniform polarization directions. The SSC detector **213** similarly detects the side scattered light including rays of nonuniform polarization directions. The forward scattered light, similar to the side scattered light, has a polarization direction that changes from the initial polarization direction according to the optical rotating power of the particles in the measurement specimen.

Similar to the side scattered light, the side fluorescent light is converged by the collecting lens **210**. The side fluorescent light that has passed through the collecting lens **210** subsequently passes through the dichroic mirror **211** and spectral filter **216**, and is then detected by the SFL detector **217**. The SFL detector **217** outputs a side fluorescent light signal (SFL) based on the detected side fluorescent light. Fluorescent light may be detected in other angle. For example, the fluorescent light can be detected in forward angle with respect to the irradiating light.

Returning now to FIG. **2**, the optical detecting section **23** outputs the forward scattered light signals (FSC), side scattered light signals (SSC), polarization scrambled light signals (PSSC), and side fluorescent light signals (SFL) to the analog signal processor **241**. The analog signal processor **241** amplifies, via an amplifier, the electrical signals from each detectors of the optical detecting section **23**, and outputs the amplified electrical signals to the A/D converter **242**.

The A/D converter **242** converts the electrical signals received from the analog signal processor **241** to digital signals, and outputs the digital signals to the digital signal processor **243**. The digital signal processor **243** performs signal processing of the digital signals received from the A/D converter **242**. Signal waveforms are obtained which correspond to the forward scattered light side scattered light, polarization scrambled side scattered light, and side fluorescent light produced when the particles pass through the flow cell **205**. That is, signal waveforms corresponding to each type of light are obtained for each particle (erythrocytes, leukocytes, epithelial cells, casts, bacteria and the like) contained in the measurement specimen. The obtained signal waveforms are stored in the memory **244**.

The CPU **25** calculates a plurality of characteristics parameters (peak level, width, area) corresponding to the forward scattered light, side scattered light, polarization scrambled side scattered light, and side fluorescent light based on the signal waveforms stored in the memory **244**.

The peak level (P) is the maximum signal level of the pulse of the signal waveform, as shown in FIG. **4**A. The width (W) is the width of the pulse of the signal waveform greater than a predetermined threshold value, as shown in FIG. **4**B. The area (A) is the area of the pulse circumscribed by the signal waveform and the line segment extending downward from the origin of the intersection of the signal waveform and predetermined threshold values, as shown in FIG. **4**C. The threshold values used in FIGS. **4**B and **4**C are suitably set according to the characteristics parameters to obtain appropriate characteristics parameters. The calculated characteristics parameters are stored in the memory **26**.

The CPU **25** transmits the calculated characteristics parameters of each particle (hereinafter referred to as "measurement data") through the communication interface **27** to the information processing device **3**. The CPU **25** receives the control signals from the information processing device **3** through the communication interface **27**, and controls each part of the measuring device **2** according to these control signals.

FIG. **5** shows the structure of the information processing device **3**.

The information processing device **3** is configured by a personal computer that includes a main body **30**, display **31**, and input section **32**. The main body **30** has a CPU **301** ROM **302**, RAM **303**, hard disk **304**, reading device **305**, image output interface **306**, I/O interface **307**, and communication interface **308**.

The CPU **301** is capable of executing a computer program stored in the ROM **302** and a computer program loaded in the RAM **303**. The RAM **303** is used when reading the computer program stored in the ROM **302** and recorded on the hard disk **304**. The RAM **303** is also used as the work area of the CPU **301** when the CPU **301** executes the computer programs.

The hard disk **304** stores an operating system and computer programs, as well as the data used when executing the computer programs that are executed by the CPU **301**. The hard disk **304** pre-stores a program **304***a* which performs the process shown in FIG. **6**, and sequentially stores measurement data received from the measuring device **2**. The reader **305** is a CD drive or DVD drive capable of reading computer programs and data recorded on a recording medium **305***a*. Note that when the program **304***a* is recorded on the recording medium **305***a*, the program **304***a* may be read from the recording medium **305***a* by the reading device **305** and stored on the hard disk **304**.

The image output interface **306** outputs image signals corresponding to the image data to the display **31**, and the display **31** displays the image based on the image signals. When the operator inputs instructions via the input section **32**, the I/O interface **307** receives the input signals. The communication interface **308** is connected to the measuring device **2**, and the CPU **301** sends and receives instruction signals and data to/from the measuring device **2** through the communication interface **308**.

FIG. **6** is a flow chart showing the processes performed by the measuring device **2** and the information processing device **3**.

When the CPU **301** of the information processing device **3** receives a measurement instruction from the operator via the input section **32** (S**101**: YES), the CPU **301** transmits a measurement start signal to the measuring device **2** (S**102**). When the CPU **25** of the measuring device **2**, on the other hand, receives a measurement start signal from the information processing device **3** (S**201**: YES), the CPU **25** controls the preparing section **22** to prepare a measurement specimen (S**202**). The CPU **25** controls the preparing section **22** to supply the prepared measurement specimen to the flow cell **205** so that the measurement specimen flows in the flow cell **205** (S**203**). Then, laser light emitted from the light source **201** irradiates the measurement specimen flowing through the flow cell **205**, and forward scattered light, side scattered light, polarization scrambled side scattered light, and side fluorescent light of each particle contained in the measurement specimen are respectively detected by the FSC detector **209**, SSC detector **213**, PSSC detector **215**, and SFL detector **217** (S**204**).

The CPU **25** then obtains the signal waveforms corresponding to each type of detected light (S**205**), and calculates the several characteristics parameters based on the obtained signal waveforms (S**206**). The CPU **25** then transmits the

several calculated characteristics parameters (measurement data) of each particle to the information processing device **3** (S**207**).

On the other hand, when the CPU **301** of the information processing device **3** receives the measurement data (S**103**: YES), the CPU **301** sets the regions A**11** and A**12** in the first scattergram which includes an axis of the width of the forward scattered light signals (FSCW) and an axis of the width of the side fluorescent light signals (FLW) (S**104**). As shown in FIG. **7**A, the CPU **301** plots each particle contained in the measurement data in the first scattergram according to the magnitude of FSCW and the magnitude of FLW.

In FIG. **7**A, the region A**11** corresponds to the total epithelial cells contained in the measurement specimen, and region A**12** corresponds to the casts contained in the measurement specimen. The particles appearing near the origin of the first scattergram, which possesses low FSCW and low FLW, are small particles such as blood cells, bacteria and the like. The CPU **301** extracts the particles, that is, the epithelial cells, in region A**11** in the first scattergram (S**105**).

In the above description particles are plotted in the first scattergram and the particles contained in region A**11** of the first scattergram are extracted. However, the regions A**11** and A**12** of the first scattergram need not necessarily be defined as a diagram or graph. Extraction of particles contained in region A**11** also may be accomplished by data processing to extract only those particles related to a specific numerical range via sorting or filtering. Similarly, the regions A**21** through A**23** of a second scattergram which will be described later need not necessarily be defined as a diagram or graph since the total number of particles contained in regions A**21** through A**23** may also be obtained by data processing such as sorting or filtering.

The CPU **301** then sets the regions A**21** through A**23** in the second scattergram which includes an axis of the width of the forward scattered light signal (FSCW) and an axis of the area of the polarization scrambled side scattered light signals (PSSCA) (S**106**). The CPU **301** plots the particles in region A**11** extracted in S**105** in the second scattergram according to the magnitude of FSCW and the magnitude of PSSCA, as shown in FIG. **7**B.

In FIG. **7**B, the region A**21** corresponds to the squamous cells, region A**22** corresponds to the renal tubular epithelial cells, and region A**23** corresponds to the ovoid fat bodies. The CPU **301** counts the particles contained in regions A**21** through A**23** of the second scattergram, that is, CPU **301** counts the squamous epithelial cells, renal tubular epithelial cells, and ovoid fat bodies.

The vertical axis PSSCA represents an amount of light polarized perpendicularly to the initial polarization direction which is proportional to the degree of polarization scrambling triggered by the particle. The ovoid fat bodies which largely contains the component to scramble polarization, compared to the squamous epithelial cells and renal tubular epithelial cells, are distributed in the region of high PSSCA. The horizontal axis FSCW represents the width of the particle. Therefore, compared to the renal tubular epithelial cells and ovoid fat bodies, the squamous epithelial cells of normally large width are distributed in the region of high FSCW. The renal tubular epithelial cells which contain not so much the component to scramble polarization compared to the ovoid fat bodies, and have small width compared to the squamous epithelial cells, are distributed in the region of low PSSCA and low FSCW. Therefore, the regions A**21** through A**23** respectively correspond to the squamous epithelial cells, renal tubular epithelial cells, and ovoid fat bodies.

The CPU **301** then shows the particle count obtained in S**107** on the display **31** (S**108**). The processes of the measuring device **2** and the information processing device **3** are thus completed.

The actual count results of a urine sample visually obtained via microscope and the count results of the urine sample obtained by the present embodiment were compared.

FIG. **8**A shows the count results of a specific urine sample visually obtained via microscope and the count results of the urine sample obtained by the present embodiment. This urine sample contained 14.0 cells/μL of squamous epithelial cells, 0.0 cells/μL renal tubular epithelial cells, and 0.0 cells/μL ovoid fat bodies by visual results. FIG. **8**B shows the second scattergram of the urine sample.

The renal tubule epithelial cells are derived from the epithelium covering the lumen from the proximal tubule, loop of Henle, distal tubule, collecting duct, to the renal papilla. Generally, if there are more than one or two renal tubular epithelial cells contained in a 1 μL urine sample, the patient from whom the urine sample was collected has a high possibility of morbid renal disease, especially at the site where renal tubule epithelial cells present. The ovoid fat bodies are fat granular cells derived from renal tubule epithelial cells. Generally, if there are more than one or two ovoid fat bodies in a 1 μL urine sample, the patient from whom the urine sample was collected has a high possibility of renal disease, for example, nephrotic syndrome.

According to the visual results of FIG. **8**A, the renal tubular epithelial cells and ovoid fat bodies contained in 1 μL urine sample is less than one. According to the results of the present embodiment, the renal tubular epithelial cells and ovoid fat bodies contained in 1 μL urine sample is also less than one, similar to the visual results. FIG. **8**B shows few particles appeared in regions A**22** and A**23** respectively corresponding to the renal tubular epithelial cells and ovoid fat bodies.

According to the present embodiment, the operator can determine there is a low possibility of renal disease in the patient based on the few renal tubular epithelial cells in the urine sample collected from the patient, similar to the visual results. Also according to the present embodiment, the operator can determine there is a low possibility the patient has renal disease based on the few ovoid fat bodies in the urine sample collected from the patient, similar to the visual results.

Since squamous epithelial cells are distributed in the mucus membrane near the external urethral orifice and many such cells are found in the urine samples of healthy persons, it is difficult to specify the site of inflammation or disease based on the number of squamous epithelial cells. However, the present embodiment improves the precision of classification of renal tubular epithelial cells and ovoid fat bodies because squamous epithelial cells can be classified from epithelial cells in the urine sample.

FIG. **8**C shows the count results obtained visually via microscope and the count results of the present embodiment concerning another urine sample which is different from the urine sample of FIGS. **8**A and **8**B. This urine sample contained 26.0 cells/μL of squamous epithelial cells, 42.0 cells/μL renal tubular epithelial cells, and 0.0 cells/μL ovoid fat bodies by visual results. FIG. **8**D shows the second scattergram of the urine sample.

According to the visual results of FIG. **8**C, the number of renal tubular epithelial cells in the 1 μL urine sample was greater than two. The number of ovoid fat bodies contained in the 1 μL urine sample was less than one. According to the results of the present embodiment, the number of renal tubular epithelial cells in the 1 μL urine sample was greater than two, and the number of ovoid fat bodies contained in the 1 μL

urine sample was less than one, similar to the visual results. FIG. **8**D shows that a large number of particles appeared in the region A**22** and few particles appeared in region A**23**. FIG. **8**D suggests that the urine sample contains many renal tubular epithelial cells and few ovoid fat bodies.

According to the present embodiment, the operator can determine there is a high possibility the patient has renal disease at the site where renal tubular epithelial cells presents based on the many renal tubular epithelial cells in the urine sample collected from the patient, similar to the visual results. Also according to the present embodiment, the operator can determine there is a low possibility the patient has renal disease such as nephrotic syndrome based on the few ovoid fat bodies in the urine sample collected from the patient, similar to the visual results.

According to the present embodiment described above, useful information is obtained pertaining to specifying type of epithelial cells contained in the measurement specimen by means of the area of the polarization scrambled side scattered light signal (PSSCA) and the width of the forward scattered light signal (FSCW). This information also may be useful for identifying the site of inflammation or disease.

More specifically, squamous epithelial cells, renal tubular epithelial cells and ovoid fat bodies can be classified by setting regions A**21** through A**23** in the second scattergram having axes of FSCW and PSSCA. The epithelial cells contained in the urine sample therefore can be finely classified as squamous epithelial cells, renal tubular epithelial cells, and ovoid fat bodies, thus providing information for identifying the site of the disease or inflammation according to the type of epithelial cell.

According to the present embodiment, the number of squamous epithelial cells, renal tubular epithelial cells, and ovoid fat bodies can be obtained and displayed by counting the number of particles in each of the regions A**21** through A**23** in the second scattergram. Based on the prevalence of renal tubular epithelial cells, the operator therefore can determine there is a high possibility the patient has renal disease at the sites of, for example, the epithelium covering the lumen from the proximal tubule, loop of Henle, distal tubule, collecting duct, to the renal papilla. The operator also can determine the patient has a high possibility of renal disease, such as nephrotic syndrome, based on the prevalence of ovoid fat bodies.

The screen D**1** shown in FIG. **9**A may be shown on the display **31** of the information processing device **3** in S**108** of FIG. **6** when the number of renal tubular epithelial cells or ovoid fat bodies is higher than a threshold number (e.g. two). Screen D**1** shows the basis for determining a high possibility of renal disease, that is the number of renal tubular epithelial cells in FIG. **9**A, and suggests the possibility of morbidity. In S**110** of FIG. **6**, the second scattergram shown in FIGS. **8**B and **8**D also may be displayed.

According to the present embodiment, the polarization direction of the laser light emitted from the light source **201** is parallel to the flow direction (Z-axis direction) of the measurement specimen flowing through the flow cell **205**. Therefore, since fluorescent light is produced in the approximate Y-axis direction when the laser light is irradiated in the X-axis positive direction relative to the particles flowing through the flow cell **205**, the side scattered light and fluorescent light can be received in approximately the same direction (Y-axis positive direction). The structure of the optical detecting section **23** therefore can be simplified. When the optical detecting section **23** is configured as described above, side fluorescent light is more efficiently detected by the SFL detector **217** arranged on the Y-axis positive side of the flow cell **205**.

According to the present embodiment, the PSSC detector **215** can efficiently detect the polarization scrambled side scattered light because the polarization filter **214** blocks the rays of side scattered light that has the same polarization direction as the initial polarization direction.

Although described by way of the above embodiments, the present invention is not limited to these embodiments and may be variously modified.

Although the light source **201** emits linearly polarized light in the above embodiment, the present invention is not limited to this configuration inasmuch as a light source module may be used which combines a generally used light source for emitting non-polarized light and a polarization filter configured to transmit only rays of single polarization direction.

Although the light source **201** is arranged within the measuring device **2** so that the polarization direction of the linearly polarized light is parallel to the direction (Z-axis direction) of the flowing measurement specimen at the laser light irradiation position on the flow cell **205**. However, the polarization direction of the laser light emitted from the light source **201** need not necessarily match the flow direction of the measurement specimen, and may be inclined relative to the direction of the flow of the measurement specimen. In this case, the travel direction of the fluorescent light moving from the particle will be distanced from the Y-axis direction compared to the above embodiment. Since the fluorescent light is efficiently detected by the SFL detector **217**, the polarization direction of the laser light emitted from the light source **201** preferably matches the flow direction of the measurement specimen as in the above embodiment.

Although the area of the polarization scrambled side scattered light signals (PSSCA) is used as one of the axes in the second scattergram in the above embodiment, other characteristics parameters can be utilized as far as that reflect the degree of polarization scrambling by the particles. For example, the peak level (PSSCP) of the polarization scrambled side scattered light may alternatively be used. In this case, whether the PSSCA or PSSCP is used as the characteristics parameter is appropriately set according to the size of the beam spot of the laser light irradiating the measurement specimen, the speed of the measurement specimen flowing through the flow cell **205**, and the amplification of the analog signal processing unit **241**.

The characteristics parameter that reflects the degree of polarization scrambling (the vertical axis of the second scattergram) may be obtained from the forward scattered light. As described above, the forward scattered light impinging the FSC detector **209** includes several rays of polarization direction. Therefore, if a half mirror is arranged on the X-axis negative direction side of the FSC detector **209** and the forward scattered light split by the half mirror then passes through a polarization filter, the optical component (polarization scrambled forward scattered light) in a polarization direction different from the initial polarization direction can be received among the forward scattered light from the particles.

Although the two characteristics parameters of the area of the polarization scrambled side scattered light signals (PSSCA) and the width of the forward scattered light signals (FSCW) are combined to classify epithelial cells in the above embodiment, the present invention is not limited to this configuration inasmuch as a single characteristics parameter reflecting the degree of collapse of the initial polarization condition may be used to classify epithelial cells. For example, instead of the second scattergram, a histogram in relation of PSSCA and number of particles can be generated. This histogram may be used as the basis of classification of

the epithelial cells. In this case, when most particles are distributed at a high position in the PSSCA, for example, it can be determined that the sample contains ovoid fat bodies. Even when most particles are distributed at a low position in the PSSCA, based on parameters such as mean value, mode value, or area of the histogram, useful information can be provided for determining whether the majority is squamous epithelial cells or renal tubular epithelial cells.

Although the width of the forward scattered light signals (FSCW) is used as the horizontal axis of the second scattergram in the above embodiment, other characteristics parameter reflecting the size of the particles also may be used. For example, the peak level of the forward scattered light signals (FSCP) or the area of the forward scattered light signals (FSCA) may be used. Or by detecting an amount of loss of light by particle which is proportional to project area of the particle may be used. Preferable characteristics parameter of the horizontal axis can be appropriately selected according to the size of the beam spot of the laser light irradiating the measurement specimen, the speed of the measurement specimen flowing through the flow cell **205**, and the amplification of the analog signal processing unit **241**.

Although the characteristics parameters reflecting the size of the particles are generated based on the forward scattered light signals detected by the optical detecting section **23** in the above embodiment, the present invention is not limited to this configuration inasmuch as the characteristics parameters also may be generated based on the signals detected by an electrical resistance type sensor provided separately in the measuring device **2**.

In the above embodiment, a light source **201** is provided in the measuring device **2** so that the polarization direction of the laser light emitted from the light source **201** is uniformed to parallel to the flow direction of the measurement specimen flowing through the flow cell **205**. In an alternative, a ½ wavelength plate may be provided on the exit side of the light source **201** so as to adjust the polarization direction of the laser light emitted from the light source **201** to be parallel to the flow direction of the measurement specimen flowing through the flow cell **205**.

Although the regions A**11**, A**12**, and A**21** through A**23** are fixed regions determined beforehand in the above embodiment, the regions may be appropriately fine tuned based on the fixed region. The position and shape of the regions A**11**, A**12**, and A**21** through A**23** are not necessarily limited to those shown in FIGS. **7**A and **7**B, and may be appropriately adjusted to positions and shapes which allow more precise extraction of squamous epithelial cells, renal tubular epithelial cells and ovoid fat bodies.

FIGS. **9**A and **9**B are modified examples of the regions A**21** through A**23** set in the second scattergram. When the intersection of the three regions is designated C in the present embodiment, the boundary of the regions A**21** and A**23** extends from intersection C in a rightward direction in FIG. **9**A, and extend from intersection C in an ascending and rightward direction in FIG. **9**B. As shown in FIGS. **9**A and **9**B, ovoid fat bodies distributed in the region of high PSSCA are included in the region S**23**, squamous epithelial cells distributed in the region of high FSCW are included in region S**21**, and renal tubular epithelial cells distributed in the region of low PSSCA and low FSCW are included in region A**22**, similar to the above embodiment.

The structure of the optical system is not necessarily limited to the structure shown in FIG. **3**, and may be configured to obtain characteristics parameters to determine the types of epithelial cells based on the degree of optical rotating power. For example, the transmission polarization direction of the polarization filter **214** need not necessarily be parallel to the X-axis direction, and may be inclined from the X-axis direction in a range of the optical rotating power can be observed.

Although urine is used as the sample in the above embodiment, the type of sample is not specifically limited insofar as the sample contained epithelial cells. For example, a sample which contains cervical cells as epithelial cells also may be analyzed. Alternatively, other liquids also may be analyzed. Such liquid may be body fluids other than blood or urine. The body fluids includes cerebrospinal fluid (CSF) filling the subarachnoid space and ventricle, pleural fluid collected in the pleural cavity, ascites fluid collected in the peritoneal cavity, pericardial fluid collected in the pericardial space, joint fluid collected in joints, synovial sac, or tendon sheaths. Peritoneal dialysis (CAPD) dialysis fluid and intraperitoneal cleaning solution are included as a type of body fluid.

Note that the present invention is not limited to the above described embodiment and may be variously modified insofar as such modifications are within the scope of the claims.

What is claimed is:

1. A urine sample analyzing method comprising:
flowing a measurement specimen prepared by mixing a urine sample and reagent through a flow cell;
irradiating epithelial cells in the measurement specimen flowing through the flow cell with linearly polarized light and thereby producing scattered light;
detecting a change of polarization condition of the scattered light produced by each of the epithelial cells; and
classifying the epithelial cells into at least two types based on the change of polarization condition.

2. The urine sample analyzing method of claim **1**, wherein
the detection of the change of polarization condition includes to detect at least a part of the scattered light produced by a polarization scrambling which the epithelial cell triggered.

3. The urine sample analyzing method of claim **1**, wherein
the detection of the change of polarization condition includes to detect a part of the scattered light having a polarization condition that differs from that of the irradiating light.

4. The urine sample analyzing method of claim **1**, wherein
the irradiating light is polarized parallel to the flow direction of the measurement specimen; and
the detection of the change of polarization condition includes to detect a part of the scattered light having a polarization direction perpendicular to that of the irradiating light.

5. The urine sample analyzing method of claim **1**, further comprising
transmitting a part of the scattered light which having a polarization direction different from that of the irradiating light to a detector via a polarization filter; and
blocking at least a part of the scattered light having a polarization direction same with that of the irradiating light via the polarization filter.

6. The urine sample analyzing method of claim **1**, further comprising
obtaining sizes of the epithelial cells based on other part of the light produced by the irradiation; and
wherein the epithelial cells are classified based on the changes of the polarization conditions and the sizes.

7. The urine sample analyzing method of claim **1**, wherein
epithelial cells are classified at least as squamous epithelial cells and renal tubular epithelial cells.

8. The urine sample analyzing method of claim **1**, wherein
epithelial cells are classified as squamous epithelial cells, renal tubular epithelial cells, and ovoid fat bodies.

9. The urine sample analyzing method of claim 8, further comprising

counting the respective classified squamous epithelial cells and renal tubular epithelial cells.

10. The urine sample analyzing method of claim 9, further comprising

displaying a message suggesting a possibility of disease when the number of the renal tubular epithelial cells is higher than a threshold.

11. The urine sample analyzing method of claim 1, further comprising

obtaining sizes of the epithelial cells based on other part of the light produced by the irradiation; and

plotting the epithelial cells on a coordinate space having an axis of a degree of the change of the polarization condition and an axis of size.

12. A urine sample analyzer comprising

a preparing section that prepares a measurement specimen by mixing a urine sample and reagent;

a flow cell through that flows the measurement specimen containing epithelial cells prepared by the preparing section;

an optical detecting section that irradiates linearly polarized light on particles in the measurement specimen flowing through the flow cell to produce scattered light, and detects a change of polarization condition of the scattered light produced by each of the epithelial cells; and

a computer programmed to classify epithelial cells contained in the measurement specimen into at least two types based on the change of the polarization condition.

13. The urine sample analyzer of claim 12, wherein

the optical detecting section detects at least a part of the scattered light produced by a polarization scrambling which the particle triggered.

14. The urine sample analyzer of claim 12, wherein

the optical detecting section detects a part of the scattered light having a polarization condition that differs from that of the irradiating light.

15. The urine sample analyzer of claim 12, wherein

the optical detecting section irradiates light polarized parallel to the flow direction of the measurement specimen, and detects scattered light polarized perpendicular to the polarization direction of the irradiating light.

16. The urine sample analyzer of claim 12, wherein

the optical detecting section comprises:

a detector arranged to detect a part of the scattered light produced by particles; and

a polarization filter arranged in the light path between the detector and the flow cell to transmit a part of the scattered light having a polarization direction different from that of the irradiating light to the detector and to block at least a part of the scattered light having a polarization direction same with that of the irradiating light.

17. The urine sample analyzer of claim 12, wherein

the optical detecting section comprises:

a first detector that converts at least a part of the scattered light which has a polarization condition different from that of the irradiating light to a first signal; and

a second detector that converts at least another part of the scattered light to a second signal indicative of size of particle,

wherein the computer is programmed to classify the epithelial cells based on the first signal and the second signal.

18. The urine sample analyzer of claim 12, wherein

the computer is programmed to classify the epithelial cells into at least squamous epithelial cells and renal tubular epithelial cells.

19. A sample analyzing method comprising

forming a sample flow of a measurement specimen containing epithelial cells;

irradiating the sample flow with a linearly polarized light to trigger a polarization scrambling; and

classifying the epithelial cells into at least squamous epithelial cells and other type of epithelial cells based on a degree of the polarization scrambling.

20. The sample analyzing method of claim 19 further comprising

preparing the measurement specimen by mixing a sample and reagent, wherein the sample is selected from urine and body fluid which is other than blood or urine.

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